



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Shizuo HATTORI et al.

Serial No.: 09/781,703 Group Art Unit: 1651

Filed: February 12, 2001 Examiner: Irene MARX

For: STABLE PQQ-DEPENDENT GLUCOSE DEHYDROGENASE COMPOSITION

DECLARATION

Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir :

I, Seiji TAKESHIMA, declare as follows:

1. I, Seiji TAKESHIMA, have following address:  
c/o TOYO BOSEKI KABUSHIKI KAISHA Tsuruga Institute of  
Biotechnology 10-24, Toyo-cho, Tsuruga-shi, Fukui, Japan.
2. I received the degree of technology from FUKUYAMA  
University in March 1991.
3. I have been employed by TOYO BOSEKI KABUSHIKI KAISHA  
since 1991, I have been engaged in research of enzyme. I  
am one of the inventors of the above-identified

application, and I am fully familiar with the subject matter thereof.

4. Specific enzyme activity of Adachi's composition in Example 4 is determined by the following calculation. Weight of stabilizing agent (g) contained in 1 liter of the composition can be calculated by: molecular weight (MW) of stabilizing agent  $\times$  molarity (M); or weight concentration (%)  $\times$  1000 ml/100 ml.

1-methoxy-5-phenazonium methysulfate: 336.36 (MW)  $\times$  0.0295 (M) = 9.923g

MTT: 414.32 (MW)  $\times$  0.0006 (M) = 0.249 g

NaN<sub>3</sub>: 65.01 (MW)  $\times$  0.0002 (M) = 0.013 g

CaCl<sub>2</sub>: 110.98 (MW)  $\times$  0.01 (M) = 0.011 g

Glutamine: 0.05 (wt%)  $\times$  1000/100 = 0.5g

Glutamic acid: 0.05 (wt%)  $\times$  1000/100 = 0.5g

Lysine: 0.05 (wt%)  $\times$  1000/100 = 0.5g

BSA: 0.2 (wt%)  $\times$  1000/100 = 2

PIPES buffer: 302.37 (MW)  $\times$  0.05 (M) = 15.119

Since the enzyme weight is small enough to be ignored, the total weight of solid content sums up to about 29.9 g. The enzyme activity is 5kU per 1 liter of composition. Accordingly, the specific enzyme activity per solid content weight figured out to be 5 kU/29.9 g = 0.167 kU/g.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: August 8, 2003

Seiji Takeshima  
Seiji TAKESHIMA